



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 37/02, C07C 261/00	A1	(11) International Publication Number: WO 92/18144 (43) International Publication Date: 29 October 1992 (29.10.92)
---	----	---

(21) International Application Number: PCT/US92/02962 (22) International Filing Date: 10 April 1992 (10.04.92) (30) Priority data: PV 1030-91 11 April 1991 (11.04.91) CS	(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent), US. Published <i>With international search report.</i>
--	--

(54) Title: SUBSTITUTED BENZHYDRYLAMINES AS HANDLES FOR SOLID PHASE PEPTIDE SYNTHESIS

(57) Abstract

The present invention is directed to a novel "handle" for solid phase peptide synthesis. The handles can be converted from a stable form to a labile form, allowing for cleavage of a peptide amide from the support after synthesis under mild conditions, and after deprotection of the amino acid side chains. The handles are based on a substituted benzhydrylamine skeleton.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MN	Mongolia
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GN	Guinea	NL	Netherlands
BJ	Benin	GR	Greece	NO	Norway
BR	Brazil	HU	Hungary	PL	Poland
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	RU	Russian Federation
CG	Congo	KP	Democratic People's Republic of Korea	SD	Sudan
CH	Switzerland	KR	Republic of Korea	SE	Sweden
CI	Côte d'Ivoire	LI	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
DE	Germany	MC	Monaco	TG	Togo
DK	Denmark			US	United States of America

- 1 -

SUBSTITUTED BENZHYDRYLAMINES AS
HANDLES FOR SOLID PHASE PEPTIDE SYNTHESIS

1. FIELD OF THE INVENTION

5 The present invention is directed to a novel "handle" for solid phase peptide synthesis. The handles can be converted from a stable form to a labile form, allowing for cleavage of a peptide from the support after synthesis under mild conditions, and
10 after deprotection of the protecting groups from the amino acid side chains. The handles are based on a substituted benzhydrylamine skeleton.

2. BACKGROUND OF THE INVENTION

15 As used in the art of peptide synthesis, the handle is defined as a bifunctional spacer that serves to link the peptide fragment or first amino acid to the polymer support. Many solid phase resins and handles are available in the art, as is described in
20 Fields and Noble, 1990, Int. J. Pept. Protein Res. 35:161-214. To be useful, handles must be stable to the reaction conditions of peptide synthesis, but when the synthesis is complete, the handle needs to allow for cleavage of the peptide from the solid support.

25 A few handles that can be converted from a stable form of handle to a labile form have been designed and synthesized. Unfortunately, these handles have found no broad application in practice. For example, the 4-benzylthiophenyl- and 4-benzylsulfonyl-handle (D.L.
30 Marshall, I.E. Liener: J. Org. Chem. 35, 867 (1970)) and sulfonamide-handle (G.W. Kenner, J.R. McDermott, R.C. Sheppard: Chem. Commun. 636, (1971)) have not found broad application. Further examples can be found in "The Peptides, Analysis, Synthesis, Biology,"
35 E. Gross, J. Meienhofer, Eds., Academic Press New York, vol. 2. p. 88 (1980), vol. 3. p. 209 (1981).

Quite recently, the handle based on the 4-alkylthiobenzyl alcohol has been disclosed (EP 274,998 and EP 274,999; Chem. Abstr. 110, 135705 (1989)) and used in peptide synthesis. After reduction of the sulfoxide moiety, the 4-alkylthiobenzyl ester is cleaved to give a peptide with free carboxyl group.

The majority of aforementioned handles have, however, certain obvious limitations concerning the amino acid residues that can be affected by the conversion step. As a rule, oxidation steps cannot be used when the peptide contains sensitive amino acids such as tryptophan, cysteine, cystine, and methionine. Another problem is possible methylation during "activation" of handle and long cleavage times which can cause the damage of peptide.

Moreover, the handles presently available are not well adapted to both Boc and Fmoc synthetic procedures.

20

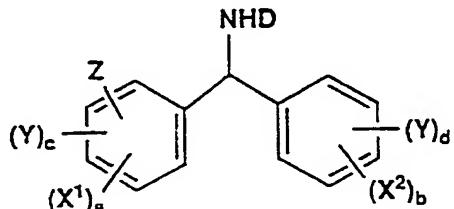
3. SUMMARY OF THE INVENTION

The instant invention is directed to substituted benzhydrylamine handles for peptide synthesis. The handles are prepared and used in acid labile form or in stable form. The stable form can be converted to an acid labile form for cleavage of the peptide from the resin.

The substituted benzhydrylamine handles are of the general formula (I):

30

35



in which a = 1 to 3, X¹ is [S]R¹ or X¹ is Z, the X¹ groups are in the ortho or para positions of the first benzene ring, and R¹ is an alkyl group and

5 in which b = 0 to 3, X² is [S]R², the X² groups are in the ortho or para positions of the second benzene ring, and R² is an alkyl group; and

 in which Z is R³, OR³ or [S]R³ in any position not occupied by X¹ unless X¹ is Z, and R³ is an alkyl group
10 comprising a reactive functional group for coupling to a solid phase support, and in which if X¹ is Z, Z is [S]R³ in an ortho or para position; and

 in which c = 0 or 1 and d = 0 or 1, Y is OR⁴, and R⁴ is an alkyl group; and

15 in which [S] is -S-, -SO- or -SO₂-; and

 in which D is H, a protecting group or an N^a-protected acyl. As used herein, "alkyl" can be a C₁ to C₁₀.

Further provided are methods for synthesis of the
20 handles of the invention. In one embodiment, hydroxy or mercapto benzophenones are reacted with ω -haloesters of alkanecarboxylic acids in the presence of fluoride ions. The benzophenone carbonyl is subsequently converted to an amine by routine
25 synthetic methods, e.g., reaction with hydroxylamine to yield an oxime, followed by reduction, e.g., with zinc, to yield benzhydrylamine, or by reductive amination, e.g., by reaction with ammonium formate. Alternatively the benzophenone can be reduced to the
30 alcohol and amidated with an N^a-protected amino acid amide.

The present invention offers distinct advantages over earlier methods of creating stable handles for peptide synthesis that can be treated after the
35 synthetic reaction is complete to make the handle labile. Once the handle is converted from stable form

to labile form, the peptide can be conveniently cleaved from a solid phase support and used. The peptide is cleaved in a carboxy amide (rather than free carboxyl) form. The oxidized handles, i.e., in the sulfoxide or sulfone form, are extraordinarily stable in acidic media, e.g., trifluoroacetic acid, as well as conditions generally used in peptide synthesis. When reduced to sulfide form, the handle is acid labile. In a preferred embodiment, the sulfoxide or sulfone is readily reduced to sulfide, to convert the stable handle to a labile form.

One particular advantage of the handles of the invention is that they allow for deprotection of the amino acid side chains prior to cleavage. Under standard solid phase synthesis, the cations generated during deprotection and cleavage are available to react with the cleaved peptide. Often reaction of these cations with the peptide results in decreased yield and purity. According to the present invention, the cations generated during deprotection can be washed away since the deprotected peptide remains on the solid support. During cleavage, the cations remain with the solid support, away from the cleaved peptide.

Another advantage of the invention is that cleavage of the peptide after reduction of the handle can be performed under much milder conditions than are generally available. For example, in a standard Boc synthesis cleavage requires treatment with the powerful acid, usually HF. The present invention thus allows a Boc synthesis with the final cleavage under much milder conditions.

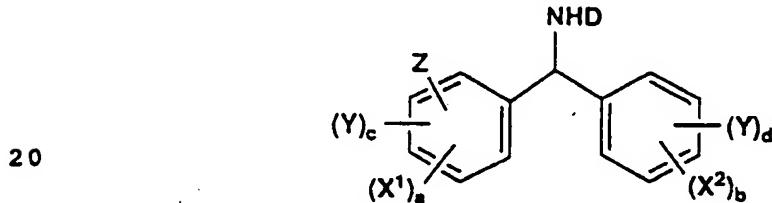
Yet a further advantage of the invention is that the handle can be used for both Fmoc and Boc synthetic strategies.

Yet another advantage of the handles of the invention is that cleavage yields a C-terminal peptide amide under less drastic conditions than are currently available.

4. DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to handles for peptide synthesis that are stable, but that can be converted to labile form for subsequent cleavage of the peptide from the solid phase support. The stability of the handles is determined by the oxidation state of an alkyl-sulfur substituent.

The substituted benzhydrylamine handles are of the general formula (I):



in which $a = 1$ to 3 , X^1 is $[S]R^1$ or X^1 is Z , the X^1 groups are in the ortho or para positions of the first benzene ring, and R^1 is an alkyl group; and

in which $b = 0$ to 3 , X^2 is $[S]R^2$, the X^2 groups are in the ortho or para positions of the second benzene ring, and R^2 is an alkyl group; and

in which Z is R^3 , OR^3 or $[S]R^3$ in any position not occupied by X^1 unless X^1 is Z , and R^3 is an alkyl group comprising a reactive functional group for coupling to a solid phase support, and in which if X^1 is Z , Z is $[S]R^3$ in an ortho or para position; and

in which $c = 0$ or 1 and $d = 0$ or 1 , Y is OR^4 , and R^4 is an alkyl group; and

in which $[S]$ is $-S-$, $-SO-$ or $-SO_2-$; and

in which D is H, a protecting group or an N"-protected acyl. As used herein, "alkyl" can be a C₁ to C₁₀.

5 As used herein, the term alkyl includes but is not limited to C₁ to about C₁₀ alkane, alkene and alkyne (i.e., saturated and unsaturated hydrocarbons). For example, an alkyl group may be methyl, ethyl, ethenyl, propyl, propenyl, propynyl, etc. The term alkyl as 10 used herein includes branched chain as well as linear chain groups, and cyclic alkyl.

As used herein, the term "handle" is defined as a bifunctional spacer. The handle has one functional group that binds to a solid phase support. The handle 15 has an second functional group that can be conjugated to an amino acid or a peptide. The handle provides for cleavage of the peptide after synthesis is complete.

As used herein, the term "solid phase support" is 20 not limited to a specific type of support. Rather a large number of supports are available and are known to one of ordinary skill in the art. Solid phase supports include silica gels, resins, derivatized plastic films, glass beads, cotton, plastic beads, 25 alumina gels. A suitable solid phase support may be selected on the basis of desired end use and suitability for various synthetic protocols. For example, for peptide synthesis, solid phase support may refer to resins such as p-methylbenzhydrylamine (pMBHA) resin (Peptides International, Louisville, KY), polystyrene (e.g., PAM-resin obtained from Bachem Inc., Peninsula Laboratories, etc.), poly (dimethylacrylamide)-grafted styrene co-divinylbenzene (e.g., POLYHIPE® resin, obtained from 35 Aminotech, Canada), polyamide resin (obtained from Peninsula Laboratories), polystyrene resin grafted

with polyethylene glycol (TentaGel®, Rapp Polymere, Tubingen, Germany) or polydimethylacrylamide resin (obtained from Milligen/Bioscience, California).

5 In one embodiment, the solid phase support may be suitable for in vivo use, i.e., it may serve as a carrier for or support for direct applications of the peptide (e.g., TentaGel, Rapp Polymere, Tubingen, Germany). In a particular embodiment, the solid phase support may be palatable and orally consumable.

10 The term "peptide" is used herein in its broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs or peptidomimetics. The subunits may be linked by peptide bonds. In 15 another embodiment, the subunit may be linked by other bonds, e.g., ester, ether, etc. As used herein the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D and L optical isomers, and amino acid 20 analogs and peptidomimetics. As used herein, a peptidomimetic is a molecule that exhibits properties similar to a peptide without having a peptide chemical structure. The peptides may comprise D-amino acids, a combination of D- and L-amino acids, and various 25 "designer" amino acids (e.g., β -methyl amino acids, α -methyl amino acids, and $\mathrm{N}\alpha$ -methyl amino acids, etc.) to convey special properties to peptides. Additionally, by assigning specific amino acids at 30 specific coupling steps, peptides with α -helices, β turns, β sheets, γ -turns, and cyclic peptides can be generated.

4.1. HANDLES

The benzhydrylamine handles of the invention are 35 substituted with at least one sulfur-containing derivative selected from the group consisting of

sulfide (SR^1), sulfoxide (SOR^1), and sulfone (SO_2R^1), in which R^1 is an alkyl group of 1 to about 10 carbon atoms. The sulfide, sulfoxide or sulfone derivative 5 is designated throughout this application as $[S]R$. The sulfur-containing group is found at the ortho position or para position on at least one benzene ring. Two sulfur-containing groups on a single benzene ring can be found at the ortho-para or both 10 ortho positions. Three sulfur containing groups can be found at the two ortho and one para positions. The benzene rings can be symmetrically substituted with sulfur-containing groups, but need not be. Preferably both benzene rings contain a sulfur-containing group 15 at the para or ortho position.

The handle further comprises a linker, Z , for attachment to a solid phase support, i.e., resin, for solid phase peptide synthesis using the handle. In one embodiment, the linker can be a sulfur-containing 20 group ($[S]R^3$) located in the ortho or para position of one benzene ring. In another embodiment, the linker can be an alkyl group (R^3), alkoxy (OR^3) group, or sulfur containing group ($[S]R^3$) located at the ortho, para or meta position, with the proviso that if Z 25 contains O or S and is in the meta position, it cannot be considered to influence the stability properties of the handle. The alkyl group on Z , R^3 , has from 1 to about 10 carbon atoms, and comprises a functional group, for example, carboxylic acid, for attachment to 30 the resin.

In a further embodiment, the handle comprises an alkoxy group (OR^4), Y , in the ortho or para positions on one or both benzene rings not occupied by a sulfur-containing group X . No group Y is found on a ring 35 that has Z in the ortho or para position when Z is OR^3 (alkoxy). The alkoxy groups can be but need not be

symmetrically arranged on the two benzene rings. If an alkoxy group (Y) is present, the number of Y groups on a single benzene ring is less than or equal to the 5 number of X groups on the ring; the total number of Y is less than or equal to the total number of X groups on the benzhydryl amine, and is preferably less. The alkyl portion of Y has 1 to about 10 carbon atoms.

Although the present invention is not limited to 10 any particular mechanism, it is believed that the properties of the handle are determined by the electron donating or electron withdrawing characteristics of substituents in the ortho or para positions of the benzene ring or rings. Thus when 15 electron donating groups, such as sulfide or alkoxy groups, are in the para or ortho positions, acidolysis of the N-C bond readily occurs because electrons can be donated to stabilize the resulting benzhydryl cation. Conversely, when an electron withdrawing 20 group such as sulfoxide or sulfone is present in the para or ortho positions, acidolysis cannot occur. Since electron donation occurs less readily from the meta position, substituents in the meta position are expected to have orders of magnitude less effect than 25 substituents in the para or ortho positions.

Substitution with both sulfoxide or sulfone substituents and alkoxy substituents results in a balance of electron withdrawing and electron donating groups. However, when the number of electron 30 withdrawing oxidized sulfur-containing groups is at least equal to and preferably greater than the number of alkoxy groups, the electron withdrawing nature of the oxidized sulfur-containing groups is expected to affect the properties of the handle to a greater 35 degree. The alkoxy groups are advantageous for cleaving the handle after reduction of the sulfur-

containing groups because of their electron donating properties.

It is believed that the stability of the oxidized handle and the acid lability of the reduced handle are enhanced by increasing the number of sulfur-containing substituents on one or both benzene rings. Thus the increased electron withdrawing nature of a handle with more than one oxidized sulfur-containing group may result in greater chemical stability of the handle. Similarly, the increased electron donating potential of more than one reduced sulfur-containing groups may facilitate cleavage under mild conditions.

Additionally, oxidation of the sulfur-containing group to yield a sulfone is expected to result in a more stable handle than oxidation to the sulfoxide because the sulfone has greater electron withdrawing properties. Since either the sulfone or the sulfoxide can be reduced to yield a sulfide, either oxidation state is suitable for handles of the invention. Presently, the sulfoxide oxidation state is preferred.

In a further embodiment, the handle can be prepared with a protecting group at the amine nitrogen. Suitable protecting groups include tert-butoxycarbonyl (Boc) and N-9-fluorenylmethyloxy carbonyl (Fmoc), to name a few. Any amine protecting group suitable for peptide synthesis is suitable. Many are described in Greene and Wuts, Protective Groups in Organic Synthesis, Wiley Interscience: New York, 1991, pp. 309-362; and Fields and Noble 1990, Int. J. Pept. Protein Res. 35: 161-214. The N-protected benzhydrylamine can be coupled to the solid support without concern for reactivity of the amine group. After the handle is coupled to the solid support, the protecting group can be removed by routine chemistry, e.g., treatment with piperidine in

dimethylformamide in the case of Fmoc, or treatment with trifluoracetic acid in the case of Boc.

In another embodiment, the handle can be prepared 5 as a benzhydrylamide, i.e., with an acyl group on the amine. Acyl groups for use in the invention include but are not limited to N^a-protected amino acids, which can be deprotected for subsequent elongation of a peptide chain. The term amino acids is used here as 10 defined above, and includes D and L amino acids, glycine, and non-naturally occurring amino acids or amino acid analogs.

When X (or Z) is found as a sulfoxide or sulfone group on the handle, the handle is extremely resistant 15 to acidic media. Reduction of a sulfoxide or sulfone to a sulfide yields a handle that is labile to acidolysis. For example, such a handle can be cleaved in trifluoroacetic acid. Thus, the present invention provides handles that are acid stable or acid labile, 20 and provides for conversion of the acid stable form to the labile form, and vice versa.

4.2. SYNTHESIS OF SUBSTITUTED BENZHYDRYLAMINES

The substituted benzhydrylamines of the present 25 invention can be prepared according to the following methods.

30 4.2.1. PREPARATION OF BENZOPHENONES

Substituted benzophenones for use as starting materials for synthesis of handles of the invention can be prepared synthetically, for example by a condensation reaction of a substituted benzoic acid 35 with a substituted benzene, i.e., acylation of benzene.

Suitable substituted benzoic acids include but are not limited to a 2-alkylthiobenzoic acid, 4-alkylthiobenzoic acid, 2,4-di(alkylthio)benzoic acid, 2,4,6-tri(alkylthio)benzoic acid, 2-(or 4-) alkylthio-4(or 2-)alkoxybenzoic acid, 2,4-(or 2,6-alkylthio)-6(or 4)-alkoxybenzoic acid, and the like. Moreover, the benzoic acid may be substituted at the 2,3 or 4 position with an alkyl group comprising a functional group for coupling to the solid phase support (i.e., Z). Special care must be taken to protect the functional group present on the alkyl group to prevent its reaction during formation of the benzophenone. For example, if the functional group is a carboxylic acid, it must be protected from reaction with thionyl chloride. Alternatively the benzoic acid can be substituted at the 2, 3 or 4 position with a hydroxy or mercapto (i.e., sulfhydryl) group. The hydroxy or mercapto group is preferably at the 2 or 4 position. If the substituted benzoic acid includes a 2 or 4 mercapto substituent, it need not include an alkylthio substituent since upon alkylation, the mercapto group will become an alkylthio group with the required properties. In a specific embodiment, the substituted benzoic acid is 2-hydroxy-4-(methylthio)benzoic acid.

Suitable substituted benzene groups include but are not limited to alkylthiobenzene (e.g., thioanisole), 1,3-dialkylthiobenzene, 1,3,5-trialkylthiobenzene, 3-alkoxy-alkylthiobenzene (e.g., 3-alkoxythioanisole), 5-alkoxy-1,3-dialkylthiobenzene and 3-alkoxy-1,5-dialkylthiobenzene. In a specific embodiment, the substituted benzene is thioanisole.

In a further embodiment, if the benzoic acid lacks an alkyl group for use as a linker, and lacks a hydroxy or mercapto group suitable for alkylation to

attach the linker, the substituted benzene can include either of these groups.

Many variations are possible for the substituted 5 benzoic acid and substituted benzene that can be used to prepare the benzophenone. Although many combinations and permutations are possible, and are contemplated by the instant invention, often the simplest starting materials are preferred, since these 10 are generally less expensive, easier to obtain, and less susceptible to undesirable side reactions. Thus, the substituted benzoic acid and substituted benzene chosen must form a benzophenone with at least one sulfur-containing substituent in a para or ortho 15 position on a benzene ring, preferably a sulfur-containing substituent on both benzene rings, and a linker or a functional group suitable for attachment of a linker.

In a specific embodiment, *infra*, a substituted 20 benzoic acid is heated in thionyl chloride to yield the corresponding benzoic acid chloride. Generally this reaction can be run by heating the benzoic acid in about two molar equivalents of thionyl chloride to about 40°C for about 10 min to about 30 min with 25 stirring. The benzoyl chloride is then dissolved (at about 0.1 to about 1M) in an aprotic solvent, e.g., ethylene dichloride, at about 40°C. This solution is mixed with an equimolar amount of a substituted benzene, e.g., thianisole, dissolved at about 0.2 to 30 about 3M in an aprotic solvent, (e.g., dichloroethane). The mixture is cooled to 0°C, and treated with an equivalent of aluminum chloride. The reaction mixture is allowed to warm to about 20°C, at 35 which point a second equivalent of AlCl₃ can be added while temperature is controlled. The reaction mixture is stirred for about 1 to 2 hours at about 40°-50°C,

and worked up by standard methods, e.g., extraction, recrystallization, etc.

It is also contemplated that a benzophenone can
5 be modified by addition of sulfur-containing groups to
the ortho or para positions, or by substitution of
sulfur for oxygen in alkoxy-substituted benzophenones.
The exchange of a sulfide for an alkoxy group occurs
10 by treatment of the hydroxy group with
chlorodimethylthiocarbamate followed by thermal
rearrangement and S-alkylation. This alternative,
however, requires use of an asymmetrically substituted
benzophenone to provide for attachment of, or
alternatively to act as, a single linker for
15 attachment to the solid phase support.

4.2.2. ALKYLATION OF HYDROXY OR MERCAPTO-SUBSTITUTED BENZOPHENONES

In the case in which the benzophenone is prepared
20 without an alkyl linking group, alkylation to add the
linking group is required. Any alkylation procedure
known in the art can be used in the practice of the
instant invention. These alkylation procedures are
accomplished by activation of an alkyl with a halogen,
25 especially bromine or chlorine, or with another good
leaving group. However, where alkylation at the ortho
position is contemplated, an agent must be added to
disrupt hydrogen bonding. In a preferred embodiment,
the agent is fluoride ion (F⁻).

30 In a specific embodiment, a substituted
benzophenone is dissolved in a polar aprotic solvent.
The benzophenone is substituted with a hydroxy or
mercapto (SH) group at the 2, 3 or 4 position on one
benzene ring. Preferably the concentration of the
35 benzophenone is 5 mM to 1 M, more preferably 50 mM to
0.5 M. The optimal concentration depends on the

choice of polar aprotic solvent, and the solubility of the benzophenone therein. Suitable solvents include but are not limited to acetonitrile, dimethyl 5 formamide, dioxane, and the like, as well as mixtures thereof.

When the hydroxy or mercapto group is in the 2-position, fluoride ions are present in the solution to facilitate the alkylation reaction. The ions can be 10 provided on an inert support. In a specific embodiment, infra, the fluoride is provided as a suspension of potassium fluoride on alumina. In another embodiment, fluoride is provided as tetraethylammonium fluoride.

15 To this solution is added an alkylating agent, e.g., an ω -haloester of alkylcarboxylic acid, which can be prepared by halogenation of the corresponding commercially available alkylcarboxylic acid. In a specific embodiment, methyl bromovalerate is added. 20 The alkylating agent is preferably added in molar excess relative to the concentration of the benzophenone, preferably greater than about a 20% excess, more preferably greater than about a 40% excess. The alkylation reaction can require at least 25 about 96 hours for completion at room temperature. The alkylated benzophenone can be purified by routine methods, e.g., chromatography or crystallization, or it can be separated from solid materials (e.g., if alumina is present) concentrated, e.g., in vacuo, and 30 used without further purification.

4.2.3. CONVERSION OF THE BENZOPHENONE TO A BENZHYDRYLAMINE

The carbonyl group of the substituted 35 benzophenones is converted to an amine group. Conversion of a carbonyl to an amine can be

accomplished by any method known in the art, including but not limited to oxime formation followed by reduction, or reductive amination, e.g., with ammonium 5 formate. In a specific embodiment, infra, the benzophenone is reacted with hydroxylamine to yield an oxime. This reaction is generally performed in a polar solvent, preferably an alcohol, e.g., ethanol, although methanol, propanol, 2-propanol, etc., can 10 also be used with the substituted benzophenone at roughly the same concentration as is used for the alkylation reaction. Anhydrous weak base, e.g., sodium acetate, can also be present. The reaction mixture is heated to reflux for about 1 to about 15 15 hours, preferably about 7 hours. The mixture can be left to stand, e.g., overnight, or worked up immediately. In work-up the reaction mixture can be filtered and concentrated in vacuo to yield a product that can be used without further purification.

20 The oxime product is dissolved in alcohol (preferably ethanol) and 25% ammonia at about a 1:3 ratio in about the same concentration as the alkylated benzophenone was used in the oxime step. To this solution is added about a 10-fold molar excess of 25 powdered zinc. The suspension is heated to 50°C and stirred for about 48 hours. The suspension is filtered and concentrated in vacuo to yield the substituted benzhydryl amine.

The benzhydrylamine obtained by reduction of the 30 oxime is sparingly soluble or insoluble in polar solvents. Moreover, when the reduction reaction is performed over zinc, zinc remains in the reaction mixture. Thus prior to oxidation of the sulfur-containing substituents (alkylsulfides) and protection 35 of the amino group, it is necessary to purify the benzhydrylamine. Purification procedures include but

are not limited to chromatography, e.g., on silica gel, recrystallization, preparative thin layer chromatography and the like.

5 In a specific embodiment, a recrystallization procedure is used. The insoluble benzhydrylamine is solubilized by adding toluene sulfonic acid, thus forming the acid salt of the amine. This reaction can be performed by dissolving the benzhydrylamine in an
10 appropriate solvent, e.g., methanol, ethanol, and the like, preferably ethanol, at about 100-500 mM, preferably at about 150 mM. A saturated solution of toluenesulfonic acid (TsOH) in the solvent can be added, preferably by a controlled addition, e.g.,
15 dropwise addition. The product is dissolved in a polar, protic solvent system that includes water (e.g., an alcohol, such as methanol, and water, 3:1 v/v), and if necessary heated, to increase solubility. After the solution has cooled to about 20°C, an
20 oxidizing agent, e.g. sodium periodate (to form the sulfoxide) or hydrogen peroxide (to form the sulfone) is added in molar excess based on equivalents of S atoms. Typically the solvent for oxidation with hydrogen peroxide is glacial acetic acid. Precipitate
25 formed during the sodium periodate oxidation is removed by filtration and the product concentrated.

4.2.4. PROTECTION OF THE AMINE GROUP

30 The present invention further provides for protection of the amine group after synthesis of the benzhydrylamine. Protection of the amine group can be important to prevent reactivity of the amine when coupling the handle to the solid support. Thus the
35 invention provides amine-protected handles.

Preferred protecting groups for the amine are Boc and Fmoc. However, the other protecting groups, such as are described in Greene and Wuts, Protective Groups in Organic Synthesis, Wiley Interscience: New York, 1991, pp. 309-362; and Fields and Noble 1990, Int. J. Pept. Protein Res. 35: 161-214 are also contemplated. The synthesis of Boc and Fmoc protected amines is straightforward and well known in the art. N^a-protected amino acids for peptide synthesis are usually protected with Boc or with Fmoc, depending on the synthetic strategy.

In a specific embodiment, infra, the amine is protected with an Fmoc protecting group. The pH of the aqueous solution containing benzhydrylamine is brought to about 9, any inorganic salts that have precipitated are removed by filtration, and protected Fmoc (e.g., Fmoc-H-N-hydroxy-succinimidyl ester) added. The Fmoc-benzhydrylamine is worked up using standard procedures to yield a product of desired purity.

In an alternative embodiment, the benzhydrylamine is protected with the Boc protecting group. Generally, this can be accomplished according to routine methods.

4.2.5. ALTERNATE ROUTE FOR PREPARING THE HANDLE

A benzophenone is prepared as described in Section 4.2.1., supra. The benzophenone can be reduced, e.g., by treatment with sodium borohydride in ethanol or other similar solvent, to yield benzhydryl alcohol. Treatment of the benzhydryl alcohol with a primary amide under acidic conditions results in substitution of the amide for the hydroxy group. This result is analogous to an acylation of a benzhydrylamine.

Preferably the amide group that is chosen is an N^a-protected amino acid, as described in Section 4.1., supra.

5

4.3. USE OF THE HANDLE FOR PEPTIDE SYNTHESIS

The handles of this invention are well suited for attaching a peptide chain to a solid phase support for peptide synthesis. The handles can be attached to any 10 amino resins via a suitable functional group, e.g., carboxylic acid, on the alkyl group. Attachment of a carboxylic acid functional group to an amine on the resin can proceed according to any of the techniques commonly used for peptide synthesis, e.g., preparation 15 of an OPfp, HOBT or other activated ester, condensation in the presence of a carbodiimide, etc. These methods are also discussed for synthesis of peptides in Section 4.3.2. Suitable solid supports for use in the invention are discussed, supra.

20 Solid phase peptide synthesis techniques are well known in the art. Simply put, an N^a-protected amino acid is activated at the α -carbonyl and coupled with the deprotected N^a of the nascent peptide-handle-solid 25 phase support. The coupling reactions may be accomplished by techniques familiar to those in the art (see, e.g., Stewart and Young, 1984, *Solid Phase Synthesis*, Second Edition, Pierce Chemical Co., Rockford, IL; Fields and Noble, 1990, "Solid phase peptide synthesis utilizing 9-fluorenylmethoxy-carbonyl amino acids," *Int. J. Pept. Protein Res.* 30 35:161-214; Geysen et al., 1987, *J. Immunol. Methods* 102:259-274). The chemistry of coupling, deprotection, and finally cleavage of the peptide from the solid phase support depends on choice of "N- 35 protecting group, which is generally tert-

butoxycarbonyl (Boc) or 9-fluorenylmethyloxycarbonyl (Fmoc).

In a preferred embodiment of peptide synthesis using handles of the invention the completeness of coupling should be assessed. Methods of assessing the completeness of coupling are well known in the art. If the coupling was not complete, the reaction should be forced to completion by a second coupling, e.g., (a) by using a higher concentration of the activated amino acid or a different activation mechanism; (b) with the addition of different or additional solvents; or (c) with the addition of chaotropic salts (see Klis and Stewart, 1990, in Peptides: Chemistry, Structure and Biology, Rivier and Marshall (eds.), EDSOM Publishers, pp. 904-906).

4.3.1. REDUCTION AND CLEAVAGE

After peptide synthesis, the peptide must be cleaved from the solid phase support. If the handle of the invention is in its reduced form, cleavage is accomplished by treatment with acid, for example TFA. Where Fmoc amino acid strategy has been employed, the cleavage will also result in deprotection of the side chains.

Alternatively, when the handle has been used in the oxidized form, either sulfoxide or sulfone, the handle can be reduced to convert it to acid labile handle. Reduction agents known in the art can be used. For example, about 1M trimethylsilylchloride/triphenylphosphine/tetrahydrofuran or more preferably 1M trimethylsilylchloride/triphenylphosphine/dichloromethane can be used to reduce the sulfoxide to the sulfide. Treatment with samarium-iodide (SmI_2) will reduce the sulfone and sulfoxide to the sulfide. In yet a further embodiment, the reduction,

deprotection and cleavage can be accomplished simultaneously by treatment with silyl bromide. In another embodiment, reduction, deprotection (of Fmoc 5 amino acids) and cleavage can be accomplished simultaneously by treatment with 1M trimethylsilylbromide/thioanisole/trifluoracetic acid.

A particular advantage of the instant invention is that the amino acid side chains can be deprotected 10 prior to cleavage. In a specific embodiment, infra, the deprotected side chains react to form a cyclic peptide prior to cleavage (see Sections 7.3 and 8., infra). By cyclizing prior to cleavage, side reactions and intermolecular bonding is avoided.

15

5. EXAMPLE: PREPARATION OF A SOLID SUPPORT
WITH A N-FMOC [2-(CARBOXYLATOBUTYL-4-OXY)-4-(METHYLSULFINYL) PHENYL]-[4'-(METHYLSULFINYL) PHENYL] METHYLAMINE

20

5.1. 4.4'-BIS(METHYLTHIO)-2-HYDROXYBENZOPHENONE

25

A solution of 2-hydroxy-4-methylthiobenzoic acid (45.00 g, 227 mmol) in thionyl chloride (50 ml, 685 mmol) was heated at 40°C for 15 min with stirring. After addition of 1,2-dichloroethane (EDC) (90 ml) the 30 solution was stirred at 50°C for 30 min under vacuum (15 mm).

35

The solid residue was dissolved in EDC (250 ml) at 40°C and added to the solution of thioanisole (26.7 ml, 227 mmol) in EDC (100 ml). The resulting mixture was cooled to 0°C and finely ground AlCl₃ (33.0 g, 247 mmol) was added portionwise within 75 min at 0-5°C. The mixture was allowed to warm to 20°C and the second equivalent of AlCl₃ (33.7 g, 253 mmol) was added within 30 min (caution: the temperature rises to 30°C and the mixture must be cooled). When addition of AlCl₃ was finished, the reaction mixture was stirred at 45-

50°C for 1.5 hr, then cooled down to 20°C and poured into mixture of ice (300 g) and conc. HCl (180 ml). The organic phase was evaporated and the aqueous layer 5 was heated at reflux for 15 min, cooled down to 20°C and extracted with EDC (4 X 150 ml). The collected organic phases were washed with water (3 x 50 ml), 10% NaHCO₃, (2 x 150 ml), water (1 x 150 ml), 1M HCl (1 x 150 ml) and water (2 x 150 ml) and then were 10 dried over MgSO₄. Removal of the solvent in vacuo gave yellow crystals which were recrystallized from EtOH (2100 ml) to give a yellow product (41.00 g, 62%), m.p. 104-106°C. Anal. Calcd for C₁₅H₁₄O₂S₂ (290.41); C 62.04%, H 4.86%, S 22.08%. Found: C 61.86%, H 4.78%, 15 S 22.06%. ¹H NMR (CDCl₃): 2.51s (3H, SCH₃); 2.54s (3H, SCH₃); 6.60-6.85m (2H, aromatic); 7.26-7.66m (5H, aromatic); 12.35s (1H, OH). MS-EI: 290(M⁺, 100); 275(20); 243(30); 228(10); 167(40); 151(30); 124(50); 105(10); 77(10); 57(10); 45(10). IR (CCl₄): ν_{CO} 20 1620cm⁻¹; ν_{OH} 3000 cm⁻¹.

5.2. 5-[3-METHYLTHIO-1-OXY-5-(4-METHYLTHIOBENZOYL)PHENYL]-PENTANOIC ACID METHYLESTER

25 A suspension of alumina (90 g, Fluka-Type 504 C, acid type) and potassium fluoride (dihydrate) (60 g) in water (600 ml) was evaporated to dryness in vacuo. The residue was dried at 135-140°C for 24h to afford 120 g of KF/Al₂O₃.

30 4,4'-Bis(methylthio)-2-hydroxybenzophenone (50.54 g, 174 mmol) was dissolved in acetonitrile (800 ml) at 60°C. The solution was rapidly cooled down to 25°C and KF/Al₂O₃ (127 g) was added all at once. To the stirred suspension methyl bromovalerate (32.4 ml, 226 35 mmol) was added portionwise within 90 hr and stirring was continued for 120 hr at which time RP HPLC

analysis indicated the reaction was complete (Vydac C-18 column, isocratic eluent 75% MeOH, 0.1% TFA, starting compound R_f = 16 min, product R_f = 7 min). The suspension was filtered, filtrate cake washed with acetone (2 x 50 ml) and the filtrate concentrated in vacuo to give yellow product which was used in the next step without further purification. Analytical sample: m.p. 61-63°C (EtOH); R_f 0.45 (petroleum ether:EtOAc - 60:40). Anal. 61-63°C (EtOH); R_f 0.45 (petroleum ether:EtOAc - 60:40). Anal. Calcd for C₂₁H₂₄O₄S₂ (404.55): C 62.35%, H 5.98%, S 15.85%. Found: C 62.20%, H 6.02%, S 15.62%. ¹H NMR (CDCl₃): 1.25-1.55m (4H, 2 x CH₂); 2.15t (2H, CH₂COO, J³ = 7.5 Hz); 2.52s (3H, SCH₃); 2.53s (3H, SCH₃); 3.64s (3H, COOCH₃); 3.90t (2H, O₂CH₂, J³ = 5Hz); 6.79d (1H, J⁴ = 1.5 Hz, aromatic); 6.88dd (1H, J³ = 8 Hz), J⁴ = 1.5 Hz, aromatic); 7.18 - 7.25 m (2H, aromatic); 7.36d (1H, J³ = 8 Hz, J⁴ = 1.5 Hz, aromatic); 7.18 - 7.25m (2H, aromatic); 7.36d (1H, J³ = 8 Hz, aromatic); 7.64 - 7.71m (2H aromatic) EI-MS: 404 (M⁺, 30); 289(20); 227(40); 167(35); 151(35); 124(20); 115(100); 83(20); 55(40).

25 5.3. 5-[3-METHYLTHIO-1-OXY-5-(4-METHYLTHIOBENZOYL)PHENYL]-PENTANOIC ACID

The above prepared crude 5-[3-methylthio-1-oxy-5-(4-methylthiobenzoyl)phenyl]pentanoic acid methylester was dissolved in dioxane (650 ml) and to this solution, 4M NaOH (131 ml, 522 mmol) and methanol (200 ml) were added. The resulting clear solution was stirred overnight at room temperature and then the organic solvents were evaporated in vacuo. The aqueous solution was extracted with ethyl acetate (EtOAc) (2x100 ml) and the aqueous layer was kept in vacuo for 30 min to remove traces of EtOAc. The

product was precipitated by slow addition of 20% H₂SO₄ (to pH 2), washed with water and dried over P₂O₅, in vacuo to give an off-white powder (50.18 g, 87%, based on starting 4,4'-bis(methylthio)-2-hydroxybenzophenone). Analytical sample: m.p. 113-114°C (EtOH); R_f 0.22 (petroleum ether:EtOAc-60:40). Anal. Calcd for C₂₀H₂₂O₄S₂ (390.52): C 61.55%, H 5.68%, S 16.42%. Found: C 61.15%, H 5.79%, S 16.39%. EI-MS: 390 (M⁺, 50); 10 290(90); 243(50); 227(40); 167(100); 151(70); 124(95), 55(60).

5.4. 2-(CARBOXYLATOBUTYL-4-OXY)-4-METHYLTHIO-4'-METHYLTHIOBENZOPHENONE OXIME

15 A suspension of 5-[3-methylthio-1-oxy-5-(4-methylthio-benzoyl)phenyl]pentanoic acid (18.00 g, 46.1 mmol), anhydrous sodium acetate (9.45 g, 115 mmol) and hydroxylamine hydrochloride (9.60 g, 138 mmol) in 96% ethanol (240 ml) was heated at reflux for 20 7 hr. After standing overnight at room temperature, the mixture was filtered and concentrated in vacuo to give a white solid, which was used without further purification in the next step. Analytical sample: m.p. 166-170°C (EtOH, isomeric mixture); R_f 0.41 25 (CHCl₃:eOH-10:1). EI-MS: 405(M⁺, 2), 387 (25); 300(45); 288(100); 242(30); 227(20); 168(30); 151(40); 124(10); 55(35).

30 5.5. [2-(CARBOXYLATOBUTYL-4-OXY)-4-(METHYTHIO)PHENYL-4'-METHYLTHIO-PHENYL]METHYLAMINE 4-TOLUENESULFONATE

Crude 2-(carboxylatobutyl-4-oxy)-4-methylthio-4'-methylthio-benzophenone oxime (minimum 46.1 mmol) prepared above was placed in a 500 ml Champagne bottle together with powdered zinc (18.37 g, 461 mmol), 35 ethanol (80 ml) and 25% ammonia (240 ml). The

suspension was magnetically stirred at 50°C for 48 hr. After filtration, the filtrate was concentrated in vacuo to give a white solid. After addition of water (300 ml) pH was adjusted to 7 with a saturated solution of toluene sulfonic acid (TsOH) in EtOH and the suspension was filtered. The off-white product was washed with water and then suspended in ethanol (300 ml) at about 60°C. To this suspension, the 5 saturated solution of TsOH in EtOH was added dropwise until the mixture turned to a clear orange solution. Standing in a refrigerator for 48 hr afforded pink crystals, which were suspended by filtration to give the product 1 (18.2 g, 70%), m.p. 178-180°C (dec); R_f 10 0.58 (n-BuOH:AcOH:H₂O-80:20:20). FAB-MS: 392(m+1); 375; 275; 227; 137. Anal calcd for C₂₇H₃₃NO₆S, (563.76); C 57.52%, H 5.9%, N 2.48%, S 17.06%. Found: C 15 57.87%, H 5.67%, N 2.40%, S 17.11%.

20 5.6. N-9-Fmoc-{[2-CARBOXYLATO-BUTYL-4-OXY)-4-(METHYLSULFINYL)-PHENYL-4'-(METHYLSULFINYL)--PHENYL]METHYLAMINE

The [2-(carboxylatobutyl-4-oxy)-4-(methylthio)phenyl-4'-methylthiophenyl)methylamine 4-toluene-sulfonate (20.00 g, 35.5 mmol) was suspended in the mixture of methanol (320 ml) and water (160 ml). The suspension was heated until the solution became clear, then chilled to 20°C and a solution of sodium periodate (15.18 g, 71 mmol) in water (140 ml) was 25 added dropwise over a period of 1 hr. The stirring was continued further for 2 hr. The sodium iodate which separated was removed by filtration and the methanol was evaporated in vacuo. The pH of remaining aqueous solution was adjusted to 9 by addition of 4 M NaOH and the solution was mixed with acetonitrile (200 ml). The mixture was filtered to remove precipitated 30 35

inorganic salts and a solution of fluorenylmethoxy-carbonyl-N-hydroxysuccinimide (Fmoc-succinimide) (12.99 g, 38.5 mmol) in acetonitrile (80 ml) was added
5 rapidly. The resultant homogenous solution was stirred at 25°C while adjusting the pH to 8.5-9 with 1M NaOH. After 2 hr no further pH change occurred and the acetonitrile was removed in vacuo. The remaining aqueous solution was diluted with water (100 ml) and extracted with ether (4 x 50 ml, discarded). The
10 aqueous part was mixed with chloroform (150 ml) and 1M H₂SO₄ was added with occasional shaking until the aqueous phase reached pH 2. The aqueous phase was then extracted with chloroform (4 x 50 ml). The
15 combined organic phases were washed with water (100 ml), dried (MgSO₄), and concentrated to give an oil which after dissolving in methanol (150 ml) and standing overnight in refrigerator afforded the product as a white solid. Concentration of mother
20 liquor and addition of ethyl acetate (50 ml) gave the second portion of the product. Yield 14.75g (65%), m.p. - 135-137°C. R_f (CHCl₃:MeOH-9:1) = 0.11. ¹H NMR (CDCl₃): 1.3-1.6m (4H, CH₂), 2.17t (2H, CH₂COO, J=8 Hz), 2.75s (3H, SOCH₃), 2.76s (3H, SOCH₃), 3.8-4.05m
25 (2H, OCH₂), 4.22t (1H, Fmoc-CH, J=8 Hz), 4.4-4.6m (2H, CH₂-Fmoc), 5.95d (1H, Ar₂CH, J=9 Hz), 6.25d (1H, NH, J=9 Hz), 7.10-7.80m (15H, aromatic). Anal Calcd for C₃₅H₃₅NO₇S₂ (645.8): C 68.10%, H 5.46%, N 2.17%, S 9.93%. Found: C 68.55%, H 5.50%, N 2.11%, S 9.89%.

30

6. EXAMPLE: ATTACHMENT OF HANDLE ONTO SUPPORT

The solution on N-Fmoc-{{[2-(carboxylatobutyl-4-oxy)-4-(methylsulfinyl)phenyl]-4'-(methylsulfinyl)-phenyl)methylamine (245 mg, 0.38 mmol), N-hydroxy-
35 benzotriazole (52 mg, 0.38 mmol), 4-dimethylamino-pyridine (5 mg, 0.038 mmol), and N,N'-diisopropyl-

carbodiimide (50 μ l, 0.38 mmol) in dimethylformamide (10 ml) was added to p-methylbenzhydrylamine (pMBHA) resin (200 mg, 0.076 mmol) (Peptides International, Louisville, KY) and the suspension was shaked overnight. The resin was washed with dimethylformamide (5 x 5 ml) and allowed to react with the solution of acetic acid (46 μ l, 0.76 mmol) and N,N'-diisopropylcarbodiimide (119 μ l, 0.76 mmol) in dimethylformamide (10 ml) for 5 hr to acetylate the remaining free amino groups. After washing with dimethylformamide (5 x 5 ml), dichloromethane (5 x 5 ml), and methanol (5 x 5 ml) the resin was dried in vacuo. The substitution estimated spectrophotometrically, was 0.34 mmol/g.

7. EXAMPLE: PREPARATION OF PEPTIDES
USING THE HANDLE

7.1. H-PHE-PRO-GLN-THR-ALA-ILE-GLY-
VAL-GLY-ALA-PRO-NH,

The above prepared resin (132 mg) was used. The general synthetic protocol was as follows: The Fmoc protecting group on the handle was removed with piperidine/dimethylformamide (1:1, v/v, 1 x 3 min, 1 x 10 min) followed by washing with dimethylformamide. Boc protecting groups were removed with mixture of trifluoroacetic acid/dichloromethane/anisole (50:50:1, 1 x 3 min, 1 x 25 min) followed by washing with dichloromethane. N-Hydroxybenzotriazole active esters were used throughout all synthesis (10 min pre-activation). The Boc protecting group was used in the cases of Gly, Val, and Ile amino acids. In the other cases, the Fmoc group was used. The coupling was monitored with bromophenol blue (Krchnak et al., 1988, Collect. Czech. Chem. Commun. 53:2542). Final cleavage from support was accomplished with the

5 mixture of 1M trimethylsilyl bromide/thioanisole/trifluoroacetic acid (2 hr, 0°C) followed by dilution with water and extraction with diethyl ether. The crude peptide was obtained in 95% yield (43 mg) and was purified on Sephadex G 25 with 1 M acetic acid as eluent. After preparative reverse phase HPLC the pure peptide was obtained in 62% yield (26.6 mg). FAB-MS: 1056 (M+1), 942, 871, 814, 715, 658, 545, 474, 391, 10 245, 217, 120. Amino acid analysis: Thr (1.00), Gln (1.02), Pro (1.97), Gly (2.00), Ala (1.95), Val (0.97), Ile (0.94), Phe (1.01).

15 7.2. BOC-TYR-ILE-GLN-ASN-CYS(C¹Z-ABU-O^TBU)-PRO-LEU-GLY-HANDLE-pMBHA RESIN

15 pMBHA resin prepared analogously as in Example 6 (400 mg, 0.052 mmol) was used for the synthesis of C¹-OXT. After each coupling, the remaining free amino groups were acetylated with the mixture of acetic acid/N-hydroxybenzotriazole/N,N'-diisopropyl-carbodiimide/N-methylimidazole. The general synthetic protocol used was the same as described in 8.1, supra. All amino acids used were Fmoc-derivatives. Amino acid analysis: Asp(0.98), Glu(1.00), Pro(0.94), 20 Gly(1.13), Cyth(0.54), Ile(0.90), Leu(1.04), 25 Tyr(0.75).

30 7.3. H₂N-CH₂-CO-TYR-ILE-GLN-ASN-CYS-PRO-LEU-GLY-NH₂(C¹-OXT)

Above prepared peptidyl-resin (Section 7.2, supra) (100 mg, 0.012 mmol) was washed with dichloromethane (3 x 5 ml) and tert-butyl protecting groups were removed with mixture of trifluoroacetic acid/dichloromethane/anisole (50:50:1, 1 x 3 min, 1 x 35 25 min), followed by washing with dichloromethane (5 x

5 ml), neutralized with 7% N,N'-diisopropylcarbodiimide/dichloromethane, washed with dichloromethane (3 x 5 ml) and finally washed with 5 N,N-dimethylformamide (3 x 5 ml). The cyclization was carried out on the support with N-hydroxybenzotriazole/N,N'-diisopropylcarbodiimide/N,N-dimethylformamide mixture for 24 hr to give a cyclized, fully protected peptide. The final cleavage 10 was accomplished with 1 M trimethylsilylchloride/thioanisole/trifluoroacetic acid mixture containing 5% of m-cresole (2 hr, 0°C). The peptide was precipitated with diethyl ether, filtered, washed with diethyl ether and purified on Sephadex G 25 (1 M 15 acetic acid) to give 10.6 mg (89%) of crude product, which was purified on preparative RP-HPLC (Vydac C18) to give 4.5 mg (38%) of a pure C¹-OXT. Amino acid analysis: Asp(0.99), Glu(1.00), Pro(0.95), Gly(0.99), Cyth(1.02), Ile(0.96), Leu(1.10), Tyr(0.93), 20 MS(FAB)=990 (M+H).

8. EXAMPLE: TWO-STEP DEPROTECTION OF
C¹-OXT PRECURSOR

Above prepared peptidyl-resin (Section 7.2, 25 supra) (100 mg, 0.012 mmol) was treated with the mixture of 1M trimethylsilylchloride/triphenylphosphine/tetrahydrofuran (2 ml) at 25°C for 2 hr. Then the resin was washed with dichloromethane (5 x 5 ml) and the peptide was cleaved from the resin with 30 the mixture of trifluoroacetic acid/m-cresole (95:5) (2.5 ml) at 25°C for 30 min. After evaporation to dryness, the peptide was precipitated with diethyl ether to give a powder which was purified on Sephadex G 25 (1 M acetic acid) followed by preparative RP-HPLC 35 (Vydac C18) to give 6.5 mg (43%) of a pure H₂N-Tyr-Ile-Gln-Asn-Cys(ClZ-Abu-OH)-Pro-Leu-Gly-NH₂. Amino acid

analysis: Asp(1.04), Glu(1.04), Pro(0.95), Gly(1.03),
Cyth(0.94), Ile(0.88), Leu(1.11), Tyr(0.85),
MS(FAB)=1176 (M+H).

5

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

20

25

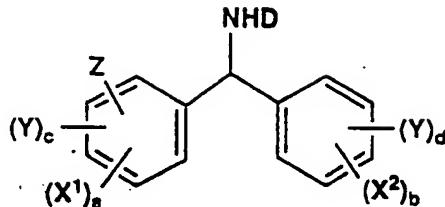
30

35

What is claimed is:

1. A handle for use in peptide synthesis of
5 the general formula (I):

10



in which a = 1 to 3, X¹ is [S]R¹, or X¹ is Z, X¹ groups
are in the ortho or para positions of the first
15 benzene ring, and R¹ is an alkyl group; and

in which b = 1 to 3, X² is [S]R², X² groups are in
the ortho or para positions of the second benzene
ring, and R² is an alkyl group; and

in which Z is R³, OR³ or [S]R³ in any position not
20 occupied by X¹ unless X¹ is Z, and R³ is an alkyl group
comprising a reactive functional group for coupling to
a solid phase support, in which if X¹ is Z, Z is [S]R³
in an ortho or para position; and

in which c = 0 or 1 and d = 0 or 1, a Y group is
25 in an ortho or para position not occupied by X¹ or X²,
Y is OR⁴, and R⁴ is an alkyl group; and

in which [S] is -S-, -SO- or -SO₂-; and

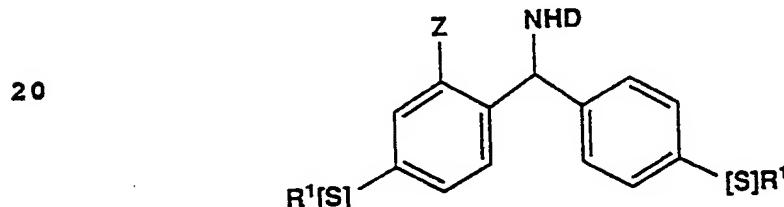
in which D is H, a protecting group or an N^{a-}
protected acyl.

30

2. The handle of claim 1 in which a = 1, X¹ is
in the para position, b = 1, and X² is in the para
position.

35 3. The handle of claim 1 in which Z is OR³ in
the ortho position.

4. The handle of claim 1 in which [S] is -S-.
5. The handle of claim 1 in which S is -SO- or
5 -SO₂-.
6. The handle of claim 1 in which P is a
protecting group.
- 10 7. The handle of claim 1 in which c = 1, d=0 and
y is in the ortho position.
8. The handle of claim 1 in which c=0, d=1, and
y is in the ortho position.
- 15 9. A handle for use in peptide synthesis of the
general formula:



25 in which Z is [S]R⁴ or OR⁴, R⁴ is an alkyl group
comprising a reactive functional group for coupling to
a solid phase support; R¹ is an alkyl group and in
which [S] is -S-, -SO-, or -SO₂-; and in which D is H,
a protecting group or an N^a-protected acylamine.

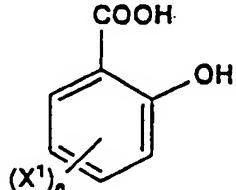
30 10. The handle of claim 9 in which Z is OR⁴.

11. A solid phase support comprising the handle
of claim 1.

35 12. A solid phase support comprising the handle
of claim 9.

13. A method for preparing a handle for use in peptide synthesis comprising the steps of:

- (a) activating a benzoic acid of the formula:

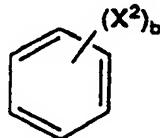


10

in which $a=1$ and X^1 is in the ortho or para position, or $a=2$ and X^1 is in the ortho and para position, X is $[S]R^1$, R^1 is an alkyl group, and $[S]$ is $-S-$, $-SO-$, or $-SO_2-$;

15

(b) reacting the benzoyl chloride prepared in step (a) with a substituted benzene of the formula:



20

in which $b=0$ to 3 , X^2 is $[S]R^2$, with the proviso that if $b > 1$, the X^2 groups are para or ortho with respect to each other, R^2 is an alkyl group, and $[S]$ is $-S-$, $-SO-$, or $-SO_2-$;

- (c) treating the mixture of step (b) with two molar equivalents of $AlCl_3$;
- (d) alkylating the $-OH$ group of the product of step (c);
- (e) reacting the product of step (d) with hydroxylamine to yield the oxime; and
- (f) reducing the oxime to yield a benzhydrylamine.

35

14. The method of claim 13 further comprising the following step:

(g) reacting the benzylhydrylamine of step
(f) with an activated FMOC at about pH 9 in aqueous
solution.

5

15. A method for preparing a resin for peptide synthesis comprising conjugating the handle of claim 1 or 9 to the resin.

10

16. The method of claim 15 further comprising oxidizing the resin-handle conjugate.

15

17. A method of peptide synthesis comprising:
(a) coupling an activated amino acid to a deprotected oxidized handle of claim 1 or 9;
(b) reducing the handle amino acid conjugate; and
(c) cleaving and deprotecting the peptide.

20

18. The method of claim 17 in which a step of deprotecting the peptide precedes the reducing step (b).

25

19. The method of claim 17 in which more than one activated amino acid is coupled to the peptide prior to the reducing step (b).

30

20. The method of claim 18 in which more than one activated amino acid is coupled to the peptide prior to the deprotecting step.

INTERNATIONAL SEARCH REPORT.

International Application No. PCT/US92/02962

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC (5): A61K 37/02; C07C 261/00 US CL : 560/27; 562/441; 525/54.11; 530/334, 341		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System	Classification Symbols	
U.S.	560/27; 562/441; 525/54.11; 530/334, 341	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁵		
CAS ONLINE, APS		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category ⁶	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X/Y, P	Chemical Abstracts, Volume 115, No. 17, issued 28 October 1991, Patek et al., "Safety-Catch Anchoring Linkage For Synthesis Of Peptide Amides By Boc/Fmoc Strategy", see page 988, column 1, abstract no. 183861S, Tetrahedron Lett., 32(31), 3891-4.	1-12/13-20
A	Journal of Organic Chemistry, Volume 41, No. 23, issued 1976, Orlowski et al., "Study of Benzhydrylamine-Type Polymers. Synthesis And Use of P-Methoxybenzhydrylamine Resin In The Solid-Phase Preparation of Peptides", pages 3701-3705. See entire article.	13-20
Y	Journal of the Chemical Society Chemical Community, Volume 5, issued 1988, Funakoshi et al., "A Modified Benzhydrylamine As A Handle Reagent For The Solid Phase Peptide Synthesis of Peptide Amides Based On Fluorenlyl Methoxy Carbonyl Method", pages 382-384. See entire article.	1-20
Y	Tetrahedron Letters, Volume 31, No. 36, issued 28 August 1990, Patek et al., "A Safety-Catch Type Of Amide Protecting Group", pages 5209-5212. See entire article.	1-20
<p>* Special categories of cited documents:¹⁵</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ²	Date of Mailing of this International Search Report ²	
30 JUNE 1992	07 JUL 1992	
International Searching Authority ¹	Signature of Authorized Officer ¹⁰	
ISA/US	BENNETT CELSA <i>J.M. Koenig Jr.</i>	



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 37/02, C07C 261/00		A1	(11) International Publication Number: WO 92/18144 (43) International Publication Date: 29 October 1992 (29.10.92)
(21) International Application Number: PCT/US92/02962 (22) International Filing Date: 10 April 1992 (10.04.92)		(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent), US.	
(30) Priority data: PV 1030-91 12 April 1991 (12.04.91)		CS	
(71)(72) Applicants and Inventors: LEBL, Michal [CS/US]; 10700 North La Reserve Dr., #16206, Tucson, AZ 85737 (US). PATEK, Marcel [CS/US]; 10700 North La Reserve Dr., #16203, Tucson, AZ 85737 (US).		Published <i>With international search report.</i>	
(74) Agent: MISROCK, S., Leslie; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).			

(54) Title: SUBSTITUTED BENZHYDRYLAMINES AS HANDLES FOR SOLID PHASE PEPTIDE SYNTHESIS

(57) Abstract

The present invention is directed to a novel "handle" for solid phase peptide synthesis. The handles can be converted from a stable form to a labile form, allowing for cleavage of a peptide amide from the support after synthesis under mild conditions, and after deprotection of the amino acid side chains. The handles are based on a substituted benzhydrylamine skeleton.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	ML	Mali
AU	Australia	FR	France	MN	Mongolia
BB	Barbados	GA	Gabon	MR	Mauritania
BE	Belgium	GB	United Kingdom	MW	Malawi
BF	Burkina Faso	GN	Guinea	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	RO	Romania
CA	Canada	IT	Italy	RU	Russian Federation
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CI	Côte d'Ivoire	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TC	Togo
DE	Germany	MC	Monaco	US	United States of America
DK	Denmark	MG	Madagascar		

THIS PAGE BLANK (USPTO)